frontiers

Supplementary Material

Mathematical modeling of proliferative immune response initiated by interactions between classical antigen presenting cells under joint antagonistic IL-2 and IL-4 signaling

Effects of cytokines IL-2 and IL-4 on T cells proliferation

Human anti-cytokine antibodies are polyclonal autoantibodies (aAbs) mainly of the IgG class and are either non-neutralizing or neutralizing or both (Schroeder Jr and Cavacini, 2010). Under physiological conditions, anti-cytokine autoantibodies may potentially play a role in the regulation of biological activities of cytokines either by neutralizing excessive cytokine production or by prolonging the half-life of cytokines in circulation by forming cytokine-antibody immune complexes (Fudala *et al.*, 2008). This potential regulatory function is evidenced by the increase in levels of anti-cytokine autoantibodies with increasing amounts of cytokines (Cappellano *et al.*, 2012). Under certain pathological conditions such as in rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE), these aAbs have been observed to rise with decrease in clinical symptoms, suggesting that anti-cytokine autoantibodies may be used as tools to monitor severity or resolution of disease (Graudal *et al*., 2002;Gupta *et al.*, 2016).

Lymphocyte populations become activated in a specific order during a T cell-dependent immune response (Andersen *et al.*, 2006). In mice injection with foreign antibodies to IgD, result in the activation of B cells by the cross-linking of their membrane (Finkelman *et al*., 1993). IgD precedes the activation of CD4+T cells (Morris *et al*., 2000). B cell activation contributes to the subsequent activation of CD4+T cells by enhancing processing of the anti-IgD antibodies and presentation of the processed antibody (Ab) to CD4+T cells specific for Ab-derived determinants (Goroff *et al.*, 1991;Finkelman *et al.*, 1993). It is worth noting that IgD is a monomeric antibody isotype that is expressed in the plasma membranes of immature B-lymphocytes. It is known that IgD signals for B cells to be activated in order for them to participate in the immune defense (Petar *et al*., 2005).

Relying on the in-vivo experiment realized in (Morris *et al*., 2000) from which we collected data upon request, the goal of our study is to assess how the concentration of cytokines IL-2 and IL-4 influences not only their productions through activation of $CD4^{+}$T cells, but also to evaluate the proliferation of CD4+T cells in terms of the concentration levels of the cytokine IL-4. We summarize the results of the study in the following. The description of other materials and methods could be found in (Morris *et al*., 2000).

Due to IL-4 enhanced anti-Ig Ab-induced B cell activation, it has been suspected that the suppressive effect of IL-4 on GαMδ-induced Ab responses might result from inhibition of the CD4+T cell response to GαMδ. The goal of the study is to investigate the effects of IL-4C treatment on T cell IL-2Rα (CD25) and cytokine expression in GαMδ-treated BALB/c mice. Mice sacrificed 4 days after GαMδ injection show a considerable increase in the percentage of CD4+T cells that express CD25. These effects on GαMδ were suppressed by IL-4 (Figure S5A). Also, GαMδ caused considerable increases in IL-2 gene expression by 3 days after injection. However, the treatment with IL-4C inhibited GαMδ-induced IL-

2 responses (Figure S5B) which systematically show a reduction of CD4^{+}T cell proliferation levels since IL-2 is the cytokine growth factor of CD4+T cells (Svetić *et al*., 1991).

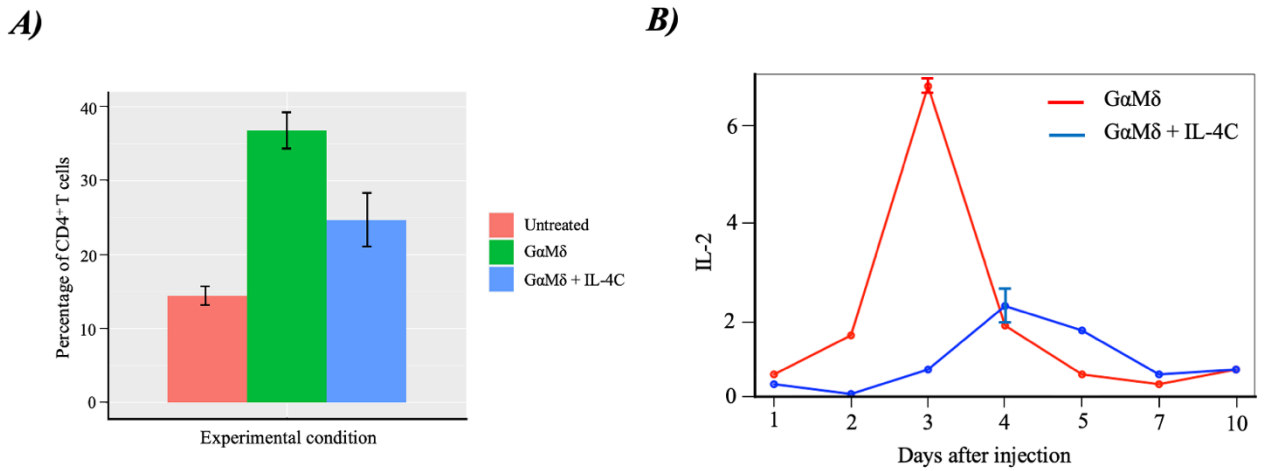

Figure S5: IL-4C inhibits GαMδ induction of CD25 expression by CD4+T cells and IL-4C inhibits gene expressions of IL-2. **A)** (1) BALB/c mice were left untreated (Untreated). (2) BALB/c mice were injected with 800 µg of GαMδ on day 0 (GαMδ treated). (3) BALB/c mice were injected with 800 µg of GαMδ + IL-4C (5 µg of IL-4 + 30 µg of anti-IL-4 mAb) on day 0 (GαMδ + IL-4C treated). Mice were sacrificed on day 4, and spleen cells were stained with FITC-labeled membrane Ab (mAb) GK1.5 (anti-CD4) and biotin-labeled mAb 7D4 (anti-CD25) followed by streptavidin-R-PE. Cells were analyzed by flow microfluorometry. Representative barplots are shown for the fluorescence of CD25 on CD4+ T cells which present arithmetic means and standard error (SE) for percentages of CD4+cells that express CD25. **B)** (1) BALB/c mice were injected with 800 µg of GαMδ on day 0 (GαMδ treated). (2) BALB/c mice were injected with 800 µg of GαMδ + IL-4C (5 µg of IL-4 + 30 µg of anti-IL-4 mAb) on day 0 (GαMδ + IL-4C treated). Mice were sacrificed 1, 2, 3, 4, 5, 7, or 10 days after injection, and splenic cytokine gene expression levels were determined by quantitative RT-PCR. Mice sacrificed on day 1, 2, 7, or 10 days after injection were associated with one experiment, and mice sacrificed on day 3, 4, or 5 days after injection were associated with another. The arithmetic means and standard error (SE) of IL-2 gene expression present a high inhibition effect of IL-4 on the production of IL-2.

Reference

Andersen, M.H., Schrama, D., Thor Straten, P., and Becker, J.C. (2006). Cytotoxic T cells. *Journal of Investigative Dermatology* 126**,** 32-41.

Cappellano, G., Orilieri, E., Woldetsadik, A.D., Boggio, E., Soluri, M.F., Comi, C., Sblattero, D., Chiocchetti, A., and Dianzani, U. (2012). Anti-cytokine autoantibodies in autoimmune diseases. *American journal of clinical and experimental immunology* 1**,** 136.

Finkelman, F.D., Madden, K.B., Morris, S., Holmes, J.M., Boiani, N., Katona, I., and Maliszewski, C. (1993). Anti-cytokine antibodies as carrier proteins. Prolongation of in vivo effects of exogenous cytokines by injection of cytokine-anti-cytokine antibody complexes. *The Journal of Immunology* 151**,** 1235-1244.

Fudala, R., Krupa, A., Stankowska, D., Allen, T.C., and Kurdowska, A.K. (2008). Anti-interleukin-8 autoantibody: interleukin-8 immune complexes in acute lung injury/acute respiratory distress syndrome. *Clinical Science* 114**,** 403-412.

Goroff, D., Holmes, J., Bazin, H., Nisol, F., and Finkelman, F. (1991). Polyclonal activation of the murine immune system by an antibody to IgD. XI. Contribution of membrane IgD cross-linking to the generation of an in vivo polyclonal antibody response. *The Journal of Immunology* 146**,** 18-25.

Graudal, N., Svenson, M., Tarp, U., Garred, P., Jurik, A., and Bendtzen, K. (2002). Autoantibodies against interleukin 1α in rheumatoid arthritis: association with long term radiographic outcome. *Annals of the rheumatic diseases* 61**,** 598-602.

Gupta, S., Tatouli, I.P., Rosen, L.B., Hasni, S., Alevizos, I., Manna, Z.G., Rivera, J., Jiang, C., Siegel, R.M., and Holland, S.M. (2016). Distinct functions of autoantibodies against interferon in systemic lupus erythematosus: a comprehensive analysis of anticytokine autoantibodies in common rheumatic diseases. *Arthritis & Rheumatology* 68**,** 1677-1687.

Morris, S.C., Gause, W.C., and Finkelman, F.D. (2000). IL-4 suppression of in vivo T cell activation and antibody production. *The Journal of Immunology* 164**,** 1734-1740.

Petar, P., Dubois, D., Rabin, B.S., and Shurin, M.R. (2005). "Immunoglobulin Titers and Immunoglobulin Subtypes," in *Measuring Immunity*. Elsevier), 158-171.

Schroeder Jr, H.W., and Cavacini, L. (2010). Structure and function of immunoglobulins. *Journal of Allergy and Clinical Immunology* 125**,** S41-S52.

Svetić, A., Finkelman, F., Jian, Y., Dieffenbach, C., Scott, D., Mccarthy, K., Steinberg, A., and Gause, W. (1991). Cytokine gene expression after in vivo primary immunization with goat antibody to mouse IgD antibody. *The Journal of Immunology* 147**,** 2391-2397.